Regulation of Hepatic Lipid and Glucose Metabolism by INSP3R1

Rachel J. Perry

Diabetes 2022;71:1834–1841 | https://doi.org/10.2337/dbi22-0003

With the rising epidemics of obesity and nonalcoholic fatty liver disease (NAFLD) and its downstream consequences including steatohepatitis, cirrhosis, and type 2 diabetes in the U.S. and worldwide, new therapeutic approaches are urgently needed to treat these devastating conditions. Glucagon, known for a century to be a glucose-raising hormone and clearly demonstrated to contribute to fasting and postprandial hyperglycemia in both type 1 and type 2 diabetes, represents an unlikely target to improve health in those with metabolic syndrome. However, recent work from our group and others' identifies an unexpected role for glucagon as a potential means of treating NAFLD, improving insulin sensitivity, and improving the lipid profile. We propose a unifying, calcium-dependent mechanism for glucagon's effects both to stimulate hepatic gluconeogenesis and to enhance hepatic mitochondrial oxidation: signaling through the inositol 1,4,5-trisphosphate receptor type 1 (INSP3R1), glucagon activates phospholipase C (PKC)/protein kinase A (PKA) signaling to enhance adipose triglyceride lipase (ATGL)-dependent intrahepatic lipolysis and, in turn, increase cytosolic gluconeogenesis by allosteric activation of pyruvate carboxylase. Simultaneously in the mitochondria, calcium transferred through mitochondria-associated membranes activates several dehydrogenases in the tricarboxylic acid cycle, correlated with an increase in mitochondrial energy expenditure and reduction in ectopic lipid. This model suggests that short-term, cyclic treatment with glucagon or other INSP3R1 antagonists could hold promise as a means to reset lipid homeostasis in patients with NAFLD.

For a century, glucagon has been considered the proverbial "ugly stepsister" of insulin. It was discovered simultaneously with insulin, when Banting and Best observed that injecting pancreatic extracts into diabetic dogs resulted in a rapid rise in blood glucose, followed by a precipitous drop (1). Most attention at the time rightly focused on the glucose-lowering properties of the pancreatic extracts, which rapidly transformed type 1 diabetes from a death sentence into a chronic disease. However, the underlying physiology of maintenance of metabolic homeostasis revealed by the immediate increase in plasma glucose in those injected with pancreatic extract was not forgotten. Studying pancreatic extracts immediately thereafter, Murlin and Kimball confirmed the existence of a hyperglycemic factor in pancreatic extracts (2) and named it "glucagon" (GLUCose-AGONist) (3). Initially, this phenotype was discounted, as it was believed to result from an increase in epinephrine following pancreatic extract injection or from a contaminant in the preparation, and glucagon's effects were largely unexplored for several decades.

Following its purification in 1949 by Sutherland et al. (4), glucagon underwent a renaissance in the 1950s. It was sequenced and commercialized and was approved by the U.S. Food and Drug Administration as a treatment for severe hypoglycemia in 1960. Simultaneously and thereafter, research clarified glucagon's position as a driver of endogenous glucose production both in vivo and in vitro, through its effects to stimulate both glycogenolysis and gluconeogenesis (5). Further work advanced the potential links between glucagon and hyperglycemia in poorly controlled diabetes: in animal models of type 1 diabetes, knocking out (6–9) or antagonizing the glucagon receptor (10–13) normalized glycemia even in the absence of endogenous insulin secretion. It should, however, be noted that mice in which the glucagon receptor was knocked down after the onset of streptozotocin-induced diabetes showed only a partial response to glucagon

Section of Endocrinology & Metabolism, Department of Internal Medicine, and Department of Cellular & Molecular Physiology, Yale School of Medicine, New Haven, CT

Corresponding author: Rachel J. Perry, rachel.perry@yale.edu

Received 25 February 2022 and accepted 13 April 2022

See accompanying articles, p. 1842 and 1852.

receptor knockdown (9), calling into question the extent to which glucagon receptor activity drives hyperglycemia on a continuing basis in existing diabetes.

Even so, based on these preclinical studies, therapeutic approaches targeting the glucagon receptor, including monoclonal antibodies, antisense oligonucleotides, and small-molecule antagonists, were subsequently advanced into phase I and II clinical trials. Unfortunately, the results have been disappointing. As reviewed recently (14), while glucagon receptor antagonism has shown some promise in terms of glucose lowering in patients with diabetes, adverse effects including hyperlipidemia and elevated transaminase concentrations—which are of particular concern in this patient population—have limited enthusiasm for the further development of glucagon-modulating interventions as an approach to antidiabetes therapy (Table 1).

Glucagon was shown by Wang et al. (15) to stimulate hepatic glucose production through activation of the INSP3 receptor. INSP3R1, a channel that permits calcium release through the endoplasmic reticulum in several cell types, is the main INSP3 expressed in liver (16). However, clinical studies have not proven to what extent changes in IP3R1 signaling per se mediate the glucose-lowering effects of glucagon receptor antagonism in certain settings. Such mechanistic studies may provide nuance regarding the known effects of glucagon to raise, and glucagon receptor antagonism to lower, blood glucose concentrations in some settings.

A strong signal was observed, in clinical trials of glucagon receptor antagonism, with regard to derangements in lipid metabolism (specifically, increases in serum LDL cholesterol and increases in liver fat) and has reinvigorated interest in exploring the impact of glucagon on both whole-body and tissue-specific energy metabolism, as reviewed by Heppner et al. (17). Indeed, a high-dose infusion of glucagon increases energy expenditure in both humans and rodents (reviewed recently in 18), presumably through an increase in mitochondrial oxidation. However, the dependence of this phenotype on or independence of this phenotype from INSP3R signaling had not been proven. These data led our group to further examine the mechanism by which glucagon simultaneously exerts its seemingly antagonistic catabolic and anabolic effects and the therapeutic implications for glucagon's effect to enhance mitochondrial energy generation.

Considering the opposite conditions under which the catabolic and anabolic programs (energy excess vs. deficit, respectively) may be advantageous, as well as their differing cellular locations (mitochondria vs. cytosol) and energetic balance (producing vs. utilizing triphosphates), we surmised that the mechanisms by which glucagon promotes gluconeogenesis and mitochondrial oxidation are likely different. While we showed that glucagon receptor activation of INSP3R1 signaling was required for both programs, the downstream mechanisms by which glucagon exerts its effects on glucose production and mitochondrial oxidation are quite different. Downstream of the glucagon receptor, PLC and PKA signaling pathways phosphorylate and activate INSP3R1. While their immediate signaling pathways are different and are outside the scope of our recent study, it appears that both the PLC and PKA signaling pathways converge on activating INSP3R1 phosphorylation. By releasing calcium into the cytosol, INSP3R1 subsequently activates cytosolic Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII), which in turn phosphorylates and thereby activates ATGL, a key rate-limiting lipolytic enzyme (19) (Fig. 1). Breakdown of intrahepatic lipids (triglycerides) in the cytosol generates both an essentially unregulated gluconeogenic substrate (glycerol) and fatty acids, which are oxidized to acetyl-CoA molecules in the mitochondria. Acetyl-CoA is an allosteric activator of pyruvate carboxylase and therefore of gluconeogenesis (20–22). We confirmed that each of these steps relies on INSP3R1-mediated calcium signaling: both INSP3R1 knockout livers in vivo and knockout hepatocytes in vitro lacked any lipolytic or gluconeogenic response to glucagon (19). In the future, it will be worthwhile to interrogate other potential targets of glucagon/IP3R1, as it is almost certain that activation of ATGL is not the only metabolic mechanism by which glucagon acts on hepatocytes to modulate hepatic lipid and glucose metabolism. Some have already been demonstrated. For example, glucagon has been shown to inhibit pyruvate kinase both transcriptionally and via phosphorylation as a result of PKA-dependent signaling (5). Because it catalyzes the conversation of phosphoenolpyruvate (PEP) to pyruvate, pyruvate kinase depletes the cytosolic pool of PEP, a key gluconeogenic precursor. Reduced pyruvate kinase expression and/or activity therefore promotes gluconeogenesis and is likely a complementary mechanism by which glucagon enhances rates of hepatic gluconeogenesis. Additionally, glucagon—or a low insulin-to-glucagon ratio—inactivates phosphofructokinase-2 in liver, thereby signaling to hepatocytes to downregulate glycolysis and enhance gluconeogenesis by depleting concentrations of fructose 2,6-bisphosphate (23). These redundant mechanisms may cooperate to ensure that glucagon-mediated gluconeogenesis will occur in the evolutionarily critical state of fasting.

A key question when placing these data in the context of the literature on glucagon biology is whether INSP3R1 signaling is responsible for the well-documented effects of glucagon on amino acid metabolism. Glucagon has been clearly shown to increase amino acid catabolism (24–27), although this effect may be more pronounced in those with nonalcoholic fatty liver disease (NAFLD) than in healthy subjects (25). Amino acids are clearly both gluconeogenic and anaplerotic substrates, and it is almost certain that amino acid catabolism contributes to both effects on metabolic physiology reported in the manuscript on which this article is based, although this mechanism was not

Table 1—Clinical trials of chronic glucagon manipulation

Ref.	Intervention	Duration	Key findings
43	Glucagon receptor antagonist (LY2409021)	12 or 24 weeks	• Dose-dependent ↓HbA_{1c} • ↓Blood glucose following mixed meals (larger reduction after 12 weeks than 24) • Dose-dependent ↑ALT
44	Glucagon receptor antagonist (LY2409021)	28 days (dose escalation)	• ↓Fasting serum glucose • ↓HbA_{1c} • Dose-dependent ↑ALT
45	Glucagon receptor antagonist (MK-0893)	12 weeks	• Dose-dependent ↓HbA_{1c} • ↑Bile acids, phytosterols
46	Glucagon receptor antagonist (MK-0893)	4 weeks	• ↓24-h weighted mean blood glucose • Trend toward ↑LDL-c, blood pressure, liver enzymes
47	Glucagon receptor antagonist (MK-0893)	12 weeks	• Dose-dependent ↓fasting blood glucose, HbA_{1c} • Dose-dependent ↑LCL-c • Dose-dependent ↑body weight
48	Glucagon receptor antagonist (MK-0893) + metformin or sitagliptin	4 weeks	• ↓Weighted mean and postprandial glucose in MK-0893 + metformin or sitagliptin vs. metformin + sitagliptin • No significant difference in LDL-c, HDL-c, or TG
49	Glucagon receptor antagonist (LGD-6972)	14 days	• ↓Fasting plasma glucose
50	Glucagon receptor antagonist (MK-3577)	4 weeks	• ↓HbA_{1c} • ↑LCL-c with MK-3577 BID
51	Glucagon receptor antagonist (LY2409021)	24 weeks	• ↓HbA_{1c} • Reversible ↑ALT • No change in lipids, weight, or blood pressure
52	Glucagon receptor antagonist (LY2409021) or sitagliptin + metformin and sulfonylurea	6 months	• ↑Hepatic fat fraction • ↑Body weight, total cholesterol, systolic blood pressure • ↓HbA_{1c} vs. placebo but not sitagliptin
53	Glucagon receptor antagonist (LY2409021)	6 weeks	• ↓HbA_{1c} • ↑ALT, AST, GGT, total cholesterol, LDL-c, TG
54	Glucagon receptor antagonist (PF-06291874)	14 or 28 days	• Dose-dependent ↓plasma glucose • Dose-dependent ↑LDL-c • Transient ↑glucogenic amino acids
55	Glucagon receptor antagonist (PF-06291874)	28 days	• ↓Mean daily and fasting plasma glucose • ↑aminotransferases • No change in LDL-c
56	Glucagon receptor antisense oligonucleotide (IONIS-$GCGR_{Rx}$) + metformin	13 or 26 weeks	• ↓HbA_{1c} • Dose-dependent ↑ALT
57	Glucagon receptor antisense oligonucleotide (IONIS-$GCGR_{Rx}$) + metformin	26 weeks	• ↓HbA_{1c} • ↓GLP-1 • Dose-dependent ↑ALT • No change in body weight, lipids, or blood pressure
58	Glucagon receptor antisense oligonucleotide (ISIS 325568)	6 weeks	• ↑Glucagon • ↓Glucagon-induced increase in plasma glucose and glucose production

Continued on p. 1837

Table 1—Continued

Ref.	Intervention	Duration	Key findings
59	Humanized IgG4 mAb (LY2786890)	28 days	• Dose-dependent ↓fasting plasma glucose • Dose-dependent ↑aminotransferases

Only studies in which treatment continued for at least 14 days are included. Studies included in this table are limited to those performed in healthy volunteers (44,49) and in subjects with type 2 diabetes (43–57,60). BID, bis in die (twice daily); GGT, γ-glutamyl transferase; GLP-1, glucagon-like peptide 1; HDL-c, HDL cholesterol; LDL-c, LDL cholesterol; mAb, monoclonal antibody; Ref., reference no.; TG, triglycerides.

tested in our study. The role of gluconeogenesis supplied by amino acids may be particularly important in the context of fasting, when muscle proteolysis is upregulated. Whether glucagon's effect to stimulate amino acid catabolism requires INSP3R1 has not been determined as of yet, but would be of great interest in future studies.

As is always the case, particularly in studying a pleotropic hormone such as glucagon, the data demonstrating that glucagon rapidly promotes hepatic gluconeogenesis must be interpreted with some degree of caution. A key evolutionary basis for glucagon must be to maintain glycemia in the fasting state, during which the duration of hyperglucagonemia far exceeds the 2-h period over which glucagon was infused in our tracer studies. Although we did not observe any impact of a 2-h infusion of glucagon on gluconeogenic protein concentrations, in the fasting state it is known that gluconeogenic protein expression does increase (28–30), likely through CREB-dependent transcription (30) in part as a result of increased glucagon concentrations (31,32). Therefore, we must not overinterpret the mechanism proposed in the recent study; it is likely that under fasting conditions, allosteric/substrate regulation of gluconeogenesis via intrahepatic lipolysis, as well as transcriptional regulation at the level of the

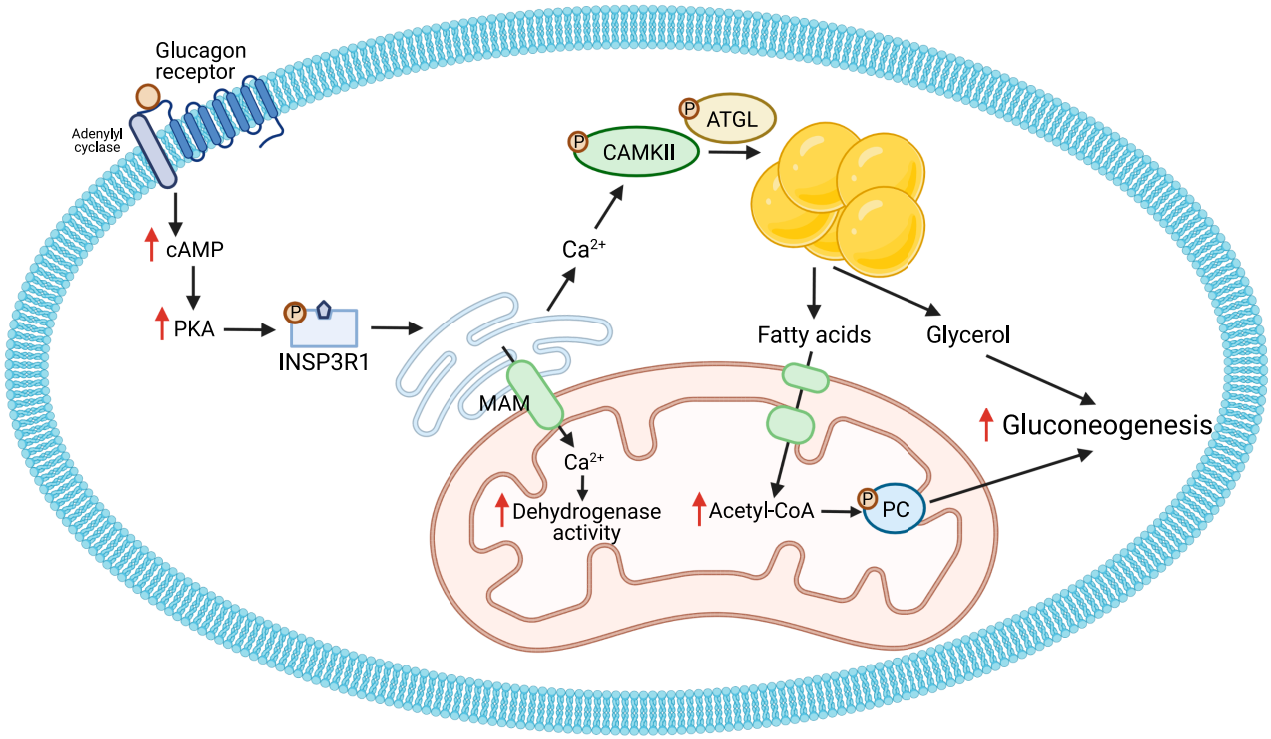

Figure 1—A simplified view of the mechanisms by which glucagon stimulates gluconeogenesis and mitochondrial oxidation, both through activation of INSP3R1 signaling. Figure created with BioRender (https://biorender.com). MAM, mitochondria-associated membrane; P, phosphorylation; PC, pyruvate carboxylase.

rate-limiting gluconeogenic enzymes, contributes to glucagon's effect to promote hepatic gluconeogenesis. Further, while this study was designed to examine the mechanisms by which glucagon promotes gluconeogenesis, it bears remembering that glucagon also has a profound effect to stimulate hepatic glycogenolysis and that under substrate-replete conditions, enhancement of glycogenolysis is likely the most physiologically important glucose-raising action of glucagon (33). Ozcan et al. (34) showed that glucagon stimulation of glycogenolysis does require CAMKII, and it is possible that INSP3R1's effect to stimulate CAMKII activity may also be responsible for glucagon's effect to promote glycogenolysis, but this hypothesis was not tested in our study.

More surprising than the mechanism by which glucagon promotes hepatic gluconeogenesis were our findings regarding glucagon's action on hepatic mitochondrial oxidation. When calcium is released from the endoplasmic reticulum as a result of INSP3R1 signaling, it not only enters the cytosol where it activates intrahepatic lipolysis, it also enters the mitochondria through mitochondria-associated membranes (Fig. 1). There, calcium activates several enzymes supplying and within the proximal part of the tricarboxylic acid cycle, including pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase (35). Accordingly, when we applied positional isotopomer nuclear magnetic resonance tracer analysis (PINTA) to examine hepatic metabolism in awake mice using a steady-state infusion of [3-^{13}C]lactate (36), we observed that glucagon acutely increases the hepatic mitochondrial oxidation rate (19). It is important to note that, although glucagon increases hepatic β-oxidation—likely as a consequence of increased fatty acid supply due to increased hepatic lipolysis—increases in hepatic acetyl-CoA concentrations cannot explain the increases in mitochondrial oxidation observed in mice treated with glucagon. The rate of tricarboxylic acid cycle flux is controlled by ADP concentrations and enzyme activity rather than substrate influx (anaplerosis). Further, acetyl-CoA is an inhibitor of pyruvate dehydrogenase activity via its effect to activate pyruvate dehydrogenase kinase, a potent inhibitor of pyruvate dehydrogenase (37). Thus, if substrate/allosteric regulation of mitochondrial oxidation were the primary regulator of this process, glucagon's lipolytic action in the liver may be expected to inhibit citrate synthase flux by increasing acetyl-CoA concentrations. This highlights the critical regulatory role for calcium in activating mitochondrial dehydrogenases and consequently the glucagon-mediated increases in hepatic mitochondrial oxidation.

A natural extension arising from our acute data demonstrating an increase in hepatic mitochondrial glucose and fatty acid oxidation with acute glucagon infusion is to ask how chronic hyperglucagonemia would affect systemic metabolism. To answer that question, we performed a chronic (3.5 week) subcutaneous infusion of glucagon in obese mice. Glucagon did not alter food intake or systemic energy expenditure (the latter likely because the dose of glucagon was selected so as to generate a physiologic, three to fivefold increase in plasma glucagon concentrations and was lower than those used in prior studies where an increase in energy expenditure was detected [18]). It is likely that glucagon-induced increases in mitochondrial oxidation were confined to certain tissues—perhaps only liver—that have a smaller bearing on whole-body energy expenditure than the largest energy producers, skeletal muscle and brown adipose tissue. These data also highlight the point that mitochondrial oxidation and ATP production are not a surrogate for total daily energy expenditure or vice versa. We showed a similar phenomenon with two mitochondrial uncouplers derived from 2,4-dinitrophenol, which increased fatty acid oxidation only in liver and not in other tissues (38,39). These uncouplers increased mitochondrial fatty acid oxidation in specific tissues, but not ATP production or whole-body energy expenditure, thereby providing an example of the decoupling, as it were, of ATP production, mitochondrial oxidation, and energy expenditure.

However, in wild-type mice, continuous glucagon infusion reduced hepatic triacylglycerol and diacylglycerol content by >50% and improved systemic insulin sensitivity, as reflected by lower plasma glucose and insulin concentrations throughout a glucose tolerance test, in both wild-type mice and rats. The data in mice were particularly striking because although the glucagon infusion was terminated prior to the glucose tolerance tests in rats, wild-type mice exhibited improved glucose tolerance despite the fact that glucagon infusion continued via subcutaneous pumps throughout the tolerance tests. In contrast, in liver-specific INSP3R1 knockout animals, glucagon had no effect on hepatic lipid content or on glucose tolerance (19).

These data would suggest that glucagon agonism is a potential therapeutic strategy for NAFLD; however, several caveats must be considered before advancing this strategy to therapeutic trials. The translatability of studies conducted exclusively in rodents lends some degree of caution in considering the therapeutic implications of the findings. Indeed, to the author's knowledge no genome-wide association studies have connected enzymes in the glucagon signaling pathway (*Gcg*, *Gcgr*, *Adcy5*, *Adcy6*, *Pnas*, *Prkaca*) with NAFLD. This may not be a fatal flaw considering the correlative nature of genome-wide association studies but may give an investigator pause before considering clinical studies using glucagon agonists.

Additionally, perhaps the phenotype observed by others that is most difficult to reconcile with our recent work on glucagon/INSP3R1-mediated mitochondrial oxidation is the finding by Feriod et al. (16) that liver-specific INSP3R1 knockout mice exhibited a modest reduction in liver

triglyceride content when fed a high-fat diet. These data are seemingly contradictory to our data demonstrating that INSP3R1 was required to mediate the effect of glucagon to reduce hepatic lipid content in obese animals: INSP3R1 knockout mice infused with glucagon exhibited >50% higher lipid content than wild-type mice infused with glucagon (19). This contradiction is particularly surprising considering that the background strain, genotype, sex, age, diet, and housing facility (though not the room in which they were housed) were all the same between our study and that of Feriod et al. Further, metabolic phenotyping was performed by the same blinded investigators through the Yale Mouse Metabolic Phenotyping Center. The most likely—if somewhat unsatisfying—explanation for these discrepancies is that IP3R1's pleotropic effects are different in the zero-to-normal range (as would be observed in IP3R1 knockouts not treated with glucagon) than in the high range (as would be observed during chronic glucagon infusions). We recognize the dubious logic of the idea that a hormone would have one effect in the low range and the opposite effect in the high range, but considering the lack of other explanations for this discrepancy, it seems the most likely possibility. Integrating our data and those of Feriod et al., it appears that glucagon plays a minimal role in basal substrate oxidation—consistent with the fact that neither study showed any difference in whole-body energy expenditure. Under ad libitum feeding conditions, circulating glucagon concentrations are low throughout the day, and it is possible that developmental compensatory mechanisms unrelated to glucagon increase hepatic mitochondrial oxidation, reducing liver triglyceride content in liver-specific INSP3R1 knockout mice. However, when glucagon is administered, INSP3R1 activation may become a primary mechanism of regulation of mitochondrial oxidation in liver. Regardless of the explanation for the seemingly divergent results of the aforementioned studies, it is clear that further investigation, both mechanistic and interventional, will be required to establish whether INSP3R1 activation may hold promise for the treatment of metabolic syndrome, NAFLD, and insulin resistance.

The most important point to be addressed before approaches targeting INSP3 signaling can move forward in clinical trials is whether INSP3/INSP3R1 signaling promotes hepatic mitochondrial oxidation—not whether approaches activating mitochondrial oxidation may be beneficial in treating NAFLD. Peroxisome proliferator–activated receptor-α agonists, including fibrates, increase fatty acid oxidation, although primarily in skeletal muscle, and as such exhibit a clear benefit in systemic lipid and glucose metabolism (40). There is substantial debate, beyond the scope of this commentary, as to whether metformin, the most commonly prescribed antidiabetes drug worldwide, is also an activator of mitochondrial β-oxidation, potentially through its hotly contested, putative effect to activate AMPK. Similarly, statins, which were developed to combat hypercholesterolemia through their ability to inhibit HMG-CoA reductase, have also been shown to enhance fatty acid oxidation (41). It is possible that glucagon may have potential in combination with one or more of these agents. Metformin may be a particularly attractive approach, as adding metformin to short-term glucagon treatment would be expected to counter the gluconeogenic effects of glucagon, while if anything enhancing the oxidative function of glucagon.

To that point, in order to attenuate metabolic dysfunction (hyperglycemia) caused by hyperglucagonemia, it will be necessary to determine the shortest possible duration of treatment with glucagon that can reduce hepatic lipid content. Glucagon will likely never be a feasible long-term strategy to treat NAFLD but may be feasible for giving patients a "head start" on normalizing hepatic lipid levels. To maintain these improvements, glucagon treatment would need to be integrated with, and then followed by, lifestyle modifications to reduce energy intake and enhance energy output. Of course, it is possible that glucagon treatment may swing the energy intake pendulum too far to be palatable: at high doses, glucagon can cause nausea and vomiting (42). Although the dose targeted for clinical trials would unquestionably be lower, these possible adverse effects would need to be monitored in clinical trials. However, low-grade nausea would be unlikely to halt the development of a drug designed to enhance mitochondrial oxidation, since several agents that cause mild nausea are commonly used for type 2 diabetes, including metformin and glucagon-like peptide 1 receptor agonists. Clinical trials will be needed to determine whether the cost-benefit balance is favorable for glucagon when applied as short-term treatment of NAFLD. Further, the potential role of INSP3R1 in mediating physiological effects of glucagon agonists or coagonists remains to be seen.

In summary, in recent work our group and others have investigated the parallel mechanisms through which glucagon promotes hepatic gluconeogenesis, by enhancing cytosolic lipolysis, β-oxidation, and pyruvate carboxylase flux, and promotes hepatic substrate oxidation, likely by activating mitochondrial dehydrogenases. Short-term treatment with glucagon or other INSP3R1 antagonists may be a promising strategy to increase hepatic mitochondrial oxidation and reverse NAFLD. As glucagon can be expected to have relatively benign therapeutic effects, at least in those without diabetes, short-term treatment with glucagon may be a viable approach to lower the activation barrier to alleviate NAFLD via lifestyle modifications. As shown in Fig. 2, these data highlight the pleotropic roles for glucagon in both anabolic and catabolic metabolism and emphasize that INSP3R1-dependent glucagon action may be important in maintaining metabolism within an appropriate range by balancing both substrate production (gluconeogenesis) and breakdown (oxidation). Glucagon agonism in combination with

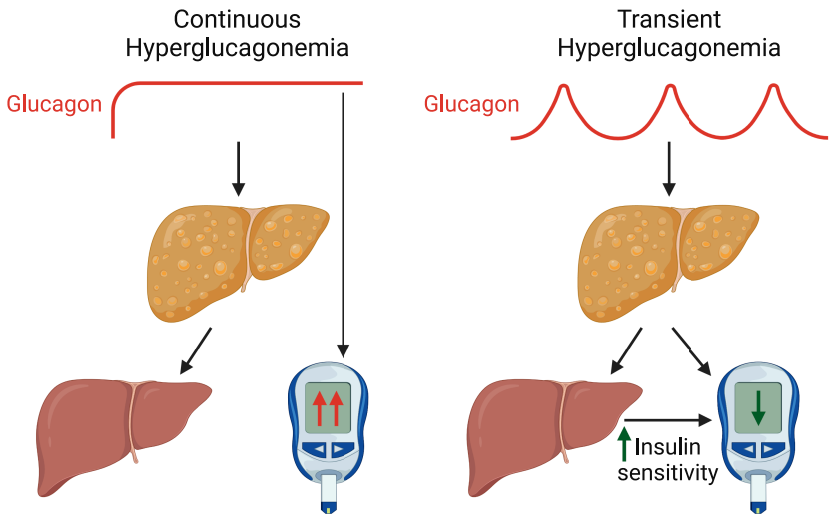

Figure 2—Proposed effect of continuous and transient hyperglucagonemia on NAFLD and blood glucose concentrations. Figure created with BioRender (https://biorender.com).

lifestyle modifications may therefore hold promise in treating NAFLD by enhancing mitochondrial oxidation.

Funding. Work in the Perry laboratory is funded by the U.S. Public Health Service (National Cancer Institute grant R37 CA258261).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Prior Presentation. Parts of this study were presented at the 82nd Scientific Sessions of the American Diabetes Association, New Orleans, LA, 3–7 June 2022.

References

1. Banting FG, Best CH. The internal secretion of the pancreas. J Lab Clin Med 1922;7:251–266
2. Kimball C, Murlin J. Aqueous extracts of pancreas III. Some precipitation reactions of insulin. J Biol Chem 1923;58:337–348
3. Murlin JR, Clough HD, Gibbs GBF, Stokes AM. Aqueous extracts of the pancreas. I. Influence on the carbohydrate metabolism of depancreatized animals. J Biol Chem 1923;56:253
4. Sutherland EW, Cori CF, Haynes R, Olsen NS. Purification of the hyperglycemic-glycogenolytic factor from insulin and from gastric mucosa. J Biol Chem 1949;180:825–837
5. Jiang G, Zhang BB. Glucagon and regulation of glucose metabolism. Am J Physiol Endocrinol Metab 2003;284:E671–E678
6. Lee Y, Wang MY, Du XQ, Charron MJ, Unger RH. Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice. Diabetes 2011;60:391–397
7. Lee Y, Berglund ED, Wang MY, et al. Metabolic manifestations of insulin deficiency do not occur without glucagon action. Proc Natl Acad Sci U S A 2012;109:14972–14976
8. Conarello SL, Jiang G, Mu J, et al. Glucagon receptor knockout mice are resistant to diet-induced obesity and streptozotocin-mediated beta cell loss and hyperglycaemia. Diabetologia 2007;50:142–150
9. Rivero-Gutierrez B, Haller A, Holland J, et al. Deletion of the glucagon receptor gene before and after experimental diabetes reveals differential protection from hyperglycemia. Mol Metab 2018;17:28–38
10. Brand CL, Jorgensen PN, Svendsen I, Holst JJ. Evidence for a major role for glucagon in regulation of plasma glucose in conscious, nondiabetic, and alloxan-induced diabetic rabbits. Diabetes 1996;45:1076–1083
11. Brand CL, Rolin B, Jorgensen PN, Svendsen I, Kristensen JS, Holst JJ. Immunoneutralization of endogenous glucagon with monoclonal glucagon antibody normalizes hyperglycaemia in moderately streptozotocin-diabetic rats. Diabetologia 1994;37:985–993
12. Rivera N, Everett-Grueter CA, Edgerton DS, et al. A novel glucagon receptor antagonist, NNC 25-0926, blunts hepatic glucose production in the conscious dog. J Pharmacol Exp Ther 2007;321:743–752
13. Wang MY, Yan H, Shi Z, et al. Glucagon receptor antibody completely suppresses type 1 diabetes phenotype without insulin by disrupting a novel diabetogenic pathway. Proc Natl Acad Sci USA 2015;112:2503–2508
14. Cheng C, Jabri S, Taoka BM, Sinz CJ. Small molecule glucagon receptor antagonists: an updated patent review (2015-2019). Expert Opin Ther Pat 2020;30:509–526
15. Wang Y, Li G, Goode J, et al. Inositol-1,4,5-trisphosphate receptor regulates hepatic gluconeogenesis in fasting and diabetes. Nature 2012;485:128–132
16. Feriod CN, Oliveira AG, Guerra MT, et al. Hepatic Inositol 1,4,5 trisphosphate receptor type 1 mediates fatty liver. Hepatol Commun 2017;1:23–35
17. Heppner KM, Habegger KM, Day J, et al. Glucagon regulation of energy metabolism. Physiol Behav 2010;100:545–548
18. Kleinert M, Sachs S, Habegger KM, Hofmann SM, Müller TD. Glucagon regulation of energy expenditure. Int J Mol Sci 2019;20:5407
19. Perry RJ, Zhang D, Guerra MT, et al. Glucagon stimulates gluconeogenesis by INSP3R1-mediated hepatic lipolysis. Nature 2020;579:279–283
20. Scrutton MC, Keech DB, Utter MF. Pyruvate carboxylase. Iv. Partial reactions and the locus of activation by acetyl coenzyme A. J Biol Chem 1965;240:574–581
21. Utter MF, Keech DB, Scrutton MC. A possible role for acetyl CoA in the control of gluconeogenesis. Adv Enzyme Regul 1964;2:49–68
22. Perry RJ, Camporez JG, Kursawe R, et al. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Cell 2015;160:745–758
23. Hers HG, Hue L. Gluconeogenesis and related aspects of glycolysis. Annu Rev Biochem 1983;52:617–653

24. Hayashi Y, Seino Y. Regulation of amino acid metabolism and alpha-cell proliferation by glucagon. J Diabetes Investig 2018;9:464–472
25. Winther-Sorensen M, Galsgaard KD, Santos A, et al. Glucagon acutely regulates hepatic amino acid catabolism and the effect may be disturbed by steatosis. Mol Metab 2020;42:101080
26. Galsgaard KD, Winther-Sorensen M, Pedersen J, et al. Glucose and amino acid metabolism in mice depend mutually on glucagon and insulin receptor signaling. Am J Physiol Endocrinol Metab 2019;316:E660–E673
27. Holst JJ, Wewer Albrechtsen NJ, Pedersen J, Knop FK. Glucagon and amino acids are linked in a mutual feedback cycle: the liver–α-cell axis. Diabetes 2017;66:235–240
28. Perry RJ, Wang Y, Cline GW, et al. Leptin mediates a glucose-fatty acid cycle to maintain glucose homeostasis in starvation. Cell 2018;172:234–248 e217
29. Lopez-Soldado I, Bertini A, Adrover A, Duran J, Guinovart JJ. Maintenance of liver glycogen during long-term fasting preserves energy state in mice. FEBS Lett 2020;594:1698–1710
30. Oh KJ, Han HS, Kim MJ, Koo SH. CREB and FoxO1: two transcription factors for the regulation of hepatic gluconeogenesis. BMB Rep 2013;46: 567–574
31. Christ B, Nath A, Bastian H, Jungermann K. Regulation of the expression of the phosphoenolpyruvate carboxykinase gene in cultured rat hepatocytes by glucagon and insulin. Eur J Biochem 1988;178:373–379
32. Yabaluri N, Bashyam MD. Hormonal regulation of gluconeogenic gene transcription in the liver. J Biosci 2010;35:473–484
33. Rix I, Nexøe-Larsen C, Bergmann NC, Lund A, Knop FK. Glucagon physiology. In Endotext. South Dartmouth, MA, MDText.com, 2019
34. Ozcan L, Wong CC, Li G, et al. Calcium signaling through CaMKII regulates hepatic glucose production in fasting and obesity. Cell Metab 2012;15:739–751
35. Traaseth N, Elfering S, Solien J, Haynes V, Giulivi C. Role of calcium signaling in the activation of mitochondrial nitric oxide synthase and citric acid cycle. Biochim Biophys Acta 2004;1658:64–71
36. Perry RJ, Peng L, Cline GW, et al. Non-invasive assessment of hepatic mitochondrial metabolism by positional isotopomer NMR tracer analysis (PINTA). Nat Commun 2017;8:798
37. Batenburg JJ, Olson MS. Regulation of pyruvate dehydrogenase by fatty acid in isolated rat liver mitochondria. J Biol Chem 1976;251:1364–1370
38. Perry RJ, Kim T, Zhang XM, et al. Reversal of hypertriglyceridemia, fatty liver disease, and insulin resistance by a liver-targeted mitochondrial uncoupler. Cell Metab 2013;18:740–748
39. Perry RJ, Zhang D, Zhang XM, Boyer JL, Shulman GI. Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats. Science 2015;347:1253–1256
40. Han L, Shen WJ, Bittner S, Kraemer FB, Azhar S. PPARs: regulators of metabolism and as therapeutic targets in cardiovascular disease. Part I: PPAR-alpha. Future Cardiol 2017;13:259–278
41. Park HS, Jang JE, Ko MS, et al. Statins increase mitochondrial and peroxisomal fatty acid oxidation in the liver and prevent non-alcoholic steatohepatitis in mice. Diabetes Metab J 2016;40:376–385
42. Ranganath L, Schaper F, Gama R, Morgan L. Mechanism of glucagon-induced nausea. Clin Endocrinol (Oxf) 1999;51:260–261
43. Kazda CM, Ding Y, Kelly RP, et al. Evaluation of efficacy and safety of the glucagon receptor antagonist LY2409021 in patients with type 2 diabetes: 12- and 24-week phase 2 studies. Diabetes Care 2016;39:1241–1249
44. Kelly RP, Garhyan P, Raddad E, et al. Short-term administration of the glucagon receptor antagonist LY2409021 lowers blood glucose in healthy people and in those with type 2 diabetes. Diabetes Obes Metab 2015;17: 414–422
45. Guan HP, Yang X, Lu K, et al. Glucagon receptor antagonism induces increased cholesterol absorption. J Lipid Res 2015;56:2183–2195
46. Ruddy M, Pramanik B, Lunceford J, et al. Inhibition of glucagon-induced hyperglycemia predicts glucose lowering efficacy of a glucagon receptor antagonist, MK-0893, in type 2 diabetes (T2DM) (Abstract). Diabetes 2011;60(Suppl. 1):A85–A86
47. Engel SS, Xu L, Andryuk PJ, et al. Efficacy and tolerability of MK-0893, a glucagon receptor antagonist (GRA), in patients with type 2 diabetes (T2DM) (Abstract). Diabetes 2011;60(Suppl. 1):309-OR
48. Engel SS, Teng R, Edwards RJ, Davies MJ, Kaufman KD, Goldstein BJ. Efficacy and safety of the glucagon receptor antagonist, MK-0893, in combination with metformin or sitagliptin in patients with type 2 diabetes mellitus (Abstract). Diabetologia 2011;54(Suppl. 1):S86
49. Vajda EG, Logan D, Lasseter K, et al. Pharmacokinetics and pharmacodynamics of single and multiple doses of the glucagon receptor antagonist LGD-6972 in healthy subjects and subjects with type 2 diabetes mellitus. Diabetes Obes Metab 2017;19:24–32
50. Engel SS, Reitman ML, Xu L, et al. Glycemic and lipid effects of the short-acting glucagon receptor antagonist MK-3577 in patients with type 2 diabetes (Abstract). Diabetes 2012;61:1037-P
51. Kazda C, Headlee S, Ding Y, et al. The glucagon receptor antagonist LY2409021 significantly lowers hba1c and is well tolerated in patients with T2DM: a 24-week phase 2 study (Abstract). Diabetes 2013;62(Suppl. 1):A29
52. Guzman CB, Zhang XM, Liu R, et al. Treatment with LY2409021, a glucagon receptor antagonist, increases liver fat in patients with type 2 diabetes. Diabetes Obes Metab 2017;19:1521–1528
53. Kazda CM, Frias J, Foga I, et al. Treatment with the glucagon receptor antagonist LY2409021 increases ambulatory blood pressure in patients with type 2 diabetes. Diabetes Obes Metab 2017;19:1071–1077
54. Kazierad DJ, Bergman A, Tan B, et al. Effects of multiple ascending doses of the glucagon receptor antagonist PF-06291874 in patients with type 2 diabetes mellitus. Diabetes Obes Metab 2016;18:795–802
55. Bergman A, Tan B, Somayaji VR, Calle RA, Kazierad DJ. A 4-week study assessing the pharmacokinetics, pharmacodynamics, safety, and tolerability of the glucagon receptor antagonist PF-06291874 administered as monotherapy in subjects with type 2 diabetes mellitus. Diabetes Res Clin Pract 2017; 126:95–104
56. Morgan ES, Tai L-J, Pham NC, et al. Antisense inhibition of glucagon receptor by IONIS-GCGR$_{Rx}$ improves type 2 diabetes without increase in hepatic glycogen content in patients with type 2 diabetes on stable metformin therapy. Diabetes Care 2019;42:585–593
57. Morgan E, Tai L, Jung SB, Geary R, Bhanot S. Low weekly doses of IONIS-GCGR$_{RX}$, a second-generation antisense glucagon receptor antagonist, caused significant improvements in glycemic control in T2DM patients on stable metformin therapy (Abstract). Diabetes 2017;66(Suppl. 1):A308–A309
58. van Dongen MG, Geerts BF, Morgan ES, et al. First proof of pharmacology in humans of a novel glucagon receptor antisense drug. J Clin Pharmacol 2015;55:298–306
59. Kelly RP, Garhyan P, Reynolds VL, et al. Glucagon receptor antibody LY2786890 reduced glucose levels in type 2 diabetes mellitus patients. Diabetes 2015;64:LB27